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Implications of Research on Assays to Characterize Thyroid Toxicants

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Many aspects of thyroid endocrinology are very well conserved across vertebrate taxa. These aspects include thyroid hormone chemistry, the mechanism of its synthesis, and the proteins involved in these processes. In addition, the system by which the hormone is delived from the thyroid gland to target cells, including transport and regulation within the hypothalamic-pituitary-thyroid (HPT) axis, and the proteins that regulate the different components of this delivery system appear to be highly conserved across the vertebrates. Finally, the receptors that mediate thyroid hormone action and the roles thyroid hormone plays are very similar among the vertebrates. Thus, the goal of this chapter is to provide a brief synopsis of the literature supporting existing screening and testing strategies in different vertebrate taxa, and to provide insight into the strengths, weaknesses, and likely changes over time. It was determined during this review that, because of the complexity of the thyroid system, it is unlikely that current *in vitro* assays for thyroid toxicity will be able to sufficiently replace *in vivo* assays for thyroid toxicants. However, the *in vitro* assays serve an important purpose in providing mode of action information and could provide potential screening tools, and should continue to be developed for use. Moreover, because *in vivo* assays are added on to preexisting reproductive or developmental screens and tests, there are no additional animals required for the *in vivo* assays. Specific *in vitro* assays were identified for development, including the thyroid receptor binding and activation assays, and *in vitro* assays to evaluate thyroid hormone action. Some *in vivo* endpoints suggested for further research included neuronal differentiation and migration, measures of histogenesis, and measures for thyroid gland thyroid hormone content, which may be more sensitive indicators of TSH stimulation. The most commonly used endpoints currently used to monitor thyroid function are thyroid hormone levels (T_3 and T_4), TSH, thyroid gland weight, and thyroid histology. Thyroid endocrinology is rapidly advancing and new discoveries will certainly warrant incorporation into future assays. The development of additional endpoints that measure thyroid hormone's actions peripheral to the HPT axis and the development of new reagents for nonmammalian vertebrate species will significantly improve the ability of today's assays to detect chemicals that disrupt the thyroid system in multiple vertebrate species. It is our hope that this series of thyroid articles will provide regulators and research scientists the information needed for each individual to identify the assays and endpoints most suited for their specific purposes.

Keywords Endocrine disruption, Screening and Testing, Thyroid Toxicity, Thyroxine

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OVERVIEW OF THE SERIES ON ASSAYS TO DETECT DISRUPTION OF THE THYROID SYSTEM ACROSS TAXA

Thyroid hormones are essential for normal development in mammals, birds, amphibians, and fishes. Therefore, chemicals in the environment that interfere with the ability of thyroid hormones to play their normal role in development could have devastating effects on wildlife or human populations, and on individuals that make up those populations. Considering the role of thyroid hormones in development, it is important to construct screens and tests for potential thyroid toxicants in any endocrine disrupter screening and testing program. These screens and tests should adequately capture the range of points within the thyroid endocrine system that may be disrupted by these toxicants. A central goal of this article is to review the current literature on thyroid endocrinology in mammals, birds, amphibians, and fish; to review and evaluate current screens and tests under consideration by various committees charged with developing a comprehensive battery that will evaluate chemicals for thyroid disruption within the context of this literature (see Table 1); and to make recommendations to consider additional assays or endpoints that address specific weaknesses in the current assays.

Several important features of the thyroid system are conserved across all taxa. The structure of T_4 and T_3 is the same in all taxa, as is the mechanism by which they are synthesized. Moreover, T_4 is the principal hormone secreted from the thyroid gland, and T_3 is the most hormonally active form in the tissue. Peripheral conversion of T_4 to T_3 contributes to controlling tissue sensitivity to thyroid hormones in all vertebrates. Thus, blood levels of T_4 represent a measure of thyroid function, and blood levels of T_3 represent a measure of peripheral deiodination of T_4. Because some animals are very small (e.g., amphibian larvae, flounder larvae), it may not always be practical to measure blood levels of hormones. Therefore, it may be necessary to develop and validate methods that utilize tissue for hormone measurements.

The functional interactions among levels of the hypothalamic-pituitary-thyroid (HPT) axis also are similar, though not identical, among vertebrates. The hypothalamus controls the pituitary, which controls the thyroid gland. Negative feedback of thyroid hormones controls the hypothalamic-pituitary axis. However, in amphibians—at least during metamorphosis—the hypothalamic peptide responsible for pituitary-thyroid activity is not the same as in other vertebrates.

TABLE 1
Existing or potential *in vivo* and *in vitro* assays

Assay name	Species	Primary thyroid-related endpoints	Target effects relevant to the thyroid system	Strengths	Weaknesses	Additional endpoints to consider for improvement
Screening assays under development/validation						
OECD TG 407 Repeated dose 28-day oral toxicity study	Rat	Total serum T_4, TSH, thyroid weight and thyroid histology.	Changes in circulating levels of TH; hypertrophy or proliferation of thyroid follicles.	Straightforward add-on; circulating levels of TH can be related to human thyroid function; follicular proliferation reflects TSH increase; thyroid histology not particularly sensitive to confounders, tumor occurrence important cancer endpoint.	Time course data lacking for compensatory changes; response to stress not characterized	Possible cardiovascular function (heart rate, blood pressure); possible body temperature. Possible liver endpoints (Malic enzyme).
OECD TG 414 Prenatal Development Toxicity Study	Rat	Total serum T_4, TSH, thyroid weight and thyroid histology.	Changes in circulating levels of TH hypertrophy or hyperplasia of thyroid follicles; possible reproductive development (e.g., testes)	Straightforward add-on; circulating levels of TH can be related to human thyroid function; follicular proliferation reflects TSH increase; thyroid histology not particularly sensitive to confounders, tumor occurrence important cancer endpoint; time-course data can be collected.	Prenatal exposure is less well studied for thyroid toxicants and for TH insufficiency. Serum volume is low, which requires pooling for assays. This reduces power. Few additional endpoints of TH action in the fetus.	
OECD 415/416 One- and Two-Gen. Reproductive Toxicity Studies	Rat	Total serum T_4, TSH, thyroid weight and thyroid histology.	Changes in circulating levels of TH hypertrophy or hyperplasia of thyroid follicles; possible reproductive development (e.g. testes)	Straightforward add-on; circulating levels of TH can be related to human thyroid function; follicular proliferation reflects TSH increase; thyroid histology not particularly sensitive to confounders , tumor occurrence important cancer endpoint; time-course data can be collected.	This is a very large study design that could capture elements of the consequences of TH disruption.	Many developmental events that are influenced by thyroid hormone could be added on. These include myelination, cortical lamination, cerebellar development. Also, possible cardiovascular development.
OECD 421/422 Reproductive/Developmental Toxicity Study	Rat	Total serum T_4, TSH, thyroid weight and thyroid histology.	Changes in circulating levels of TH hypertrophy or hyperplasia of thyroid follicles; possible reproductive development (e.g., testes)	Straightforward add-on; circulating levels of TH can be related to human thyroid function; follicular proliferation reflects TSH increase; thyroid histology not particularly sensitive to confounders, tumor occurrence important cancer endpoint; time-course data can be collected.	This is a very large study design that could capture elements of the consequences of TH disruption.	Many developmental events that are influenced by thyroid hormone could be added on. These include myelination, cortical lamination, cerebellar development. Also, possible cardiovascular development.
OECD 426 Developmental Neurotoxicity Study		Total serum T_4, TSH, thyroid weight and thyroid histology.	Changes in circulating levels of TH hypertrophy or hyperplasia of thyroid follicles; possible reproductive development (e.g. testes)	Straightforward add-on; circulating levels of TH can be related to human thyroid function; follicular proliferation reflects TSH increase; thyroid histology not particularly sensitive to confounders, tumor occurrence important cancer endpoint; time-course data can be collected.	This is a very large study design that could capture elements of the consequences of TH disruption.	Many developmental events that are influenced by thyroid hormone could be added on. These include myelination, cortical lamination, cerebellar development. Also, possible cardiovascular development.

(Continued on next page)

TABLE 1
Existing or potential *in vivo* and *in vitro* assays *(Continued)*

Assay name	Species	Primary thyroid-related endpoints	Target effects relevant to the thyroid system	Strengths	Weaknesses	Additional endpoints to consider for improvement
Amphibian Metamorphosis Assay (21-Day Assay with initiation at NF stage 51)	*Xenopus laevis*	Hind limb length; thyroid gland histology; whole body length; developmental stage; mortality.	Normal, delayed, or accelerated metamorphosis from tadpole to frog.	May be more sensitive than tail resorption alone; more comprehensive than other Tier I screens for thyroid; relatively short; can accommodate other biochemical and molecular biomarkers.	Toxicant metabolism is unknown across taxa.	T_4 levels
			Potential *in vitro* screening assays			
In vitro receptor binding	Isolated recombin-ant receptors from any vertebrate	T_3 binding to receptor	May be important mechanism by which some toxicants could interfere with thyroid signaling	Solid state binding assays available; low rate of false positive; appropriate for high through-put	Receptor binding not fully characterized as a mechanism; high false negative; no metabolic activation; solubility	
Receptor activation using recombinant receptors (from any vertebrate)	Various types of cell lines	Functional assay to define pharmacology	Tissue end organ effects of T_3	Can determine agonist or antagonist properties; system can be manipulated, optimized, etc.; readily adapted to high through-put	Limited metabolic activity; cell wall (yeast)	
Thyroid peroxidase (TPO) using lactoperoxidase		Iodine organification	Iodine organification	Sensitive; unlikely to produce false positives; *In vitro* uses fewer animals; could be adapted to high-through-put application	No rodent or human TPO available; high false negative due to specificity; only one of many MOAs that affect hormone levels.	
Binding to serum proteins (TTR, TBG)	Rat, human, others by design	Displacement of $T_4$from proteins; potentially reduce serum T_4;	May be a mechanism by which some chemicals cause serum T_4 reduction; potentially may reduce T_4 uptake into tissue including brain.	Well-characterized; can be modified for high through-put; may be predictive of chemicals that alter fetal T_4	Many other MOAs affect serum hormones in addition to this; TTR knock-outs do not support relevance to adverse effects.	
Deiodinase	Frog, fish, possibly mammal	Conversion of T_4 to T_3 (outer ring deiodinase) or reverse T_3 (inner ring deiodination)	Potentially a mechanism by which tissues regulate their sensitivity to thyroid hormone	Well characterized assay; important endpoint for tailored tests	Not a single assay (three types); tissue and species differences in deiodinases	
Glucuronidation	Rat, others as available	T_4 glucuronidate	T_4deactivation, reduction of circulating levels	Well-characterized; *in vivo* exposure, *ex vivo* assay; inducible; not as sensitive to diurnal rhythm or stress	Very specific; high false negative; somewhat laborious	
GH_3 cell assay	Rat	Growth/proliferation; normal morphology of cell signals; can be constructed to identify agonist/antagonist	Local tissue effect of T_3	High through-put adaptability; uses fewer animals; can detect agonist or antagonist activity	Specific for TR binding; high false negative	
			Current tests (with the option to add thyroid endpoints)			
Mammalian one- or two-generation	Rat/ mouse	T_4/TSH levels, thyroid weight and histopathology being considered as add-on	Hormone levels and histopathology would provide potential measure of thyroid dysfunction during development	Would provide at least some thyroid specific endpoints; provides a postnatal developmental hormone profile; doesn't use additional animals	Does not provide endpoints of specific hormone effects in tissue	(PND4, PND21, Adult) In addition to hormone levels and thyroid histopathology: serum binding proteins; serum Tg; thyroid gland hormone content; cortical lamina (BrdU in utero); cerebellar histology (P5-15); granule cell apoptosis (P5-10); oligo number or anterior commissure area; heart development.

Tests currently being developed						
Fish two gen	Fat head minnow, medaka, zebrafish, sheeps-head minnow	T_4 levels (whole body/serum/tissue), thyroid weight and histopathology	Thyroid status	Nonmammalian test; thyroid function effects over time/development stages	May be insensitive to thyroid toxicants; tissue measures may be inaccurate or laborious; few TSH methods (may require development); T_3 not currently included	TSH, T_3 measurements; deiodinase assay; gill chloride
Avian Two-Generation Assay	Japanese quail	Circulating T_4, T_3, TSH, thyroid weight, thyroid histology, bone length, skeletal endpoints; thyroid gland hormone content; body weight/growth rate	Developmental profile of thyroid function, assay of thyroid hormone-sensitive tissues (skeleton); HPT axis activation	Doesn't require sacrifice; relatively inexpensive, simple, quick; easily validated; new information indicates gland TH content is sensitive and reliable.	T_4 and T_3 are highly variable; no TSH assays; histopath is labor intensive. Body weight very insensitive	
Amphibian Growth and Reproduction Test	*X. (Silurana) tropicalis*	Hindlimb length, thyroid gland histology, whole body length, developmental stage (rate)	Normal, delayed or accelerated development from tadpole to frog	May be more sensitive than tail resorption alone; more comprehensive than other Tier I screens for thyroid; relatively short; can accommodate other biochemical and molecular biomarkers. The battery may also provide advanced developmental, sexual differentiation, and other reproductive endpoints in addition to the previously mentioned thyroid-related endpoints (i.e., the test is all-inclusive).	Toxicant metabolism is unknown across taxa.	TSH, T_4, and T_3, steroid hormones, and aromatase
Consideration for research and development						
Avian embryo assay	Japanese quail	Toxicant application to external air cell membrane; thyroid endpoints during embryonic development and 1-day chick including gland hormone measurements; histopathology; skeletal x-ray	Developmental endpoints of thyroid function and thyroid hormone action	Developmental times may be more sensitive to thyroid-specific toxicants	Unknown sensitivity to thyroid hormone or thyroid toxicants	
Larval fish assay	Larval fish	Transition from larval to juvenile form; potential large number of morphological changes associated with transformation (e.g., gut, fins, mouth) Development/growth, hormone content, histopathology	Normal, delayed, or accelerated morphogenesis from larval to adult form	Developing larval fish have largely been ignored in thyroid studies but may prove to be highly susceptible to thyroid disruption	Techniques will need to be refined for thyroid analyses of extremely small fish. Relevance to other taxa, especially mammals, is unknown. This assay requires further development and refinement, standardization and validation	
Flounder metamorphosis assay	Flounder	Transition from planktonic to benthic; potential large number of morphological changes associated with metamorphosis (e.g., eye migration, pigment asymmetry, stomach formation)	Normal, delayed, or accelerated morphogenesis from larva to juvenile	Straightforward morphological and behavioral endpoints, reflecting integrated effects of thyroid hormones	Does not consider other components of the fish thyroid cascade, such as central T_4 production (brain-pituitary-thyroid axis). Relevance to other taxa, especially mammals, is unknown. This assay requires further development and refinement, standardization and validation	

Thus, while the general functionality of the system is the same among the vertebrates, there are differences in specific molecules that must be considered.

Thyroid hormone does not regulate the same developmental or physiological endpoints in all organs within a single animal, and the same is true across all vertebrates. Thus, thyroid hormones control events in the metamorphosing amphibian that are likely to be different in human development. However, within the context of thyroid toxicology, these different endpoints can be viewed as ways of testing the hypothesis that a specific chemical can interfere with thyroid hormone action. For example, the drug propylthiouracil (PTU) can reduce blood levels of thyroid hormone in both amphibians and in rodents. However, PTU-induced reductions in blood levels of thyroid hormone will not affect the same endpoints in the two species, but will similarly be indicative of an antithyroid agent.

All known thyroid toxicants have been identified by their ability to alter serum levels of thyroid hormones (Brucker-Davis, 1998) because this is currently the only definition of thyroid toxicity. It has been reasonably argued that serum concentrations of thyroid hormones should be an indicator of all thyroid toxicants (DeVito et al., 1999). Hormone levels will reveal thyroid toxicants that interfere with thyroid function (by any mechanism), thyroid hormone metabolism (by any mechanism), or TR activation (in principle). For example, chemicals that inhibit thyroperoxidase would reduce T_4 synthesis and would suppress serum T_4. Likewise, chemicals that increase thyroid hormone metabolism and clearance from serum (e.g., UDPGT inducers) would cause a reduction in serum T_4 or at least an increase in serum TSH (to maintain normal T_4 levels). Finally, chemicals that interfere with TR activation should alter the negative feedback action of thyroid hormone at the hypothalamus and pituitary, thereby causing a change in serum thyroid hormone levels. Thus, hormone levels are and will remain important indicators of thyroid toxicity.

However, changes in serum hormone concentrations do not indicate the specific effects that these changes will have on an organism. Thus, while a strong argument can be made for using serum hormone concentrations and thyroid weight/histology as the sole indicators of thyroid toxicity, their value in risk assessment is complicated because not all toxicants produce changes in serum T_3, T_4, TSH and thyroid histology that are consistent with the idealized model of thyroid physiology based on the effects of PTU (OECD, 2006). Thus, some toxicants will produce dose-responses that do not follow this idealized model (see Zoeller, 2006b), and there will be confusion about when changes in these endpoints leads to "compensation" versus a clearly harmful effect.

As reviewed in this document, new research indicates that endpoints can be developed that will likely prove to be sensitive indicators of adverse effects of thyroid hormone insufficiency and of thyroid toxicity. These endpoints represent measures of thyroid hormone action, both in development and in the adult. While we await development of new measures for these assays by the scientific community, changes can be made immediately to improve the sensitivity of the current assays. For example, alterations in thyroid hormone levels during the early postnatal period are currently not accounted for in any of the existing assays; these measurements should be incorporated into the screens. Specifically, T_4 levels in normal rat pups are in the range of 0.5 to 1.0 μg/dl on PND 4 (Goldey et al., 1995; Zoeller et al., 2000), rising to 8 to 12 μg/dl on PND 15, then declining to adult levels of approximately 3 μg/dl by PND 21. Thus, chemicals that affect serum hormone levels on P15, but not on P21, would not be captured in an experimental protocol in which P21 was the only time that serum thyroid hormone levels were measured. Incidentally, the radioimmunoassay used extensively in toxicological research is a commercial kit based on human serum and calibrated for human serum T_4 levels that are slightly higher than for rats. This kit has a lowest standard of 1 (or in some kits 2) μg/dl. Because serum samples that do not have T_4 levels above that of the lowest standard cannot be interpreted, measurements in the literature should be carefully evaluated because many of these are below the detectability of the assay kit used. Moreover, although the structures of thyroid hormones (T_4 and T_3) are identical among all vertebrates, the composition of the serum differs among animals, which may confound the assay.

Finally, the combined use of *in vivo* and *in vitro* screens for thyroid toxicants requires careful consideration. The number of targets of thyroid toxicity is high—in the thyroid gland alone, toxicants are known to interact directly with the sodium-iodide symporter, the transport protein Pendrin, the peroxidase enzyme, and enzymes of thyroglobulin catabolism. Each of these points of disruption produces a slightly different dose-response effect on serum hormone levels. In addition, thyroid hormone synthesis depends on the dual oxidase enzymes for the local production of hydrogen peroxide and there may be environmental chemicals that interact with these proteins. There is evidence that TSH signaling in the thyroid gland can be regulated by iodocompounds that may also be targets of disruption. Finally, once thyroid hormone is released into the blood stream, a large number of factors can influence its ability to play its role in development and physiology. Serum binding proteins are known targets of toxicants, though it remains unclear if these effects mediate observed actions of these toxicants. Likewise, toxicants can induce metabolizing enzymes in the liver (UDPGTs). However, for thyroid hormone to gain access into cells, they must interact with specific transporters, the OATPs and MTC8 proteins. These are clearly physiologically important, but we have no knowledge of their vulnerability to specific toxicants. And finally, T_4 must be converted to T_3 to exert an effect on the thyroid hormone receptor. This large number of regulatory points is difficult to imagine being incorporated into a battery of *in vitro* screens. Perhaps more importantly, even if each one of these steps could be separately evaluated in an *in vitro* assay, given the limited knowledge we have at this time on each aspect of the thyroid system, only an *in vivo* assay could monitor the way these points of regulation respond to a perturbation by

toxicant exposure. For example, we might easily show that a low dose of toxicant exposure can inhibit the TPO enzyme. But to what extent must TPO be inhibited before thyroid hormone synthesis is inhibited. Moreover, to what extent must thyroid hormone synthesis be inhibited before circulating levels of thyroid hormone are compromised? And finally, to what extent must thyroid hormone levels be reduced (or increased) and for what duration, before an adverse outcome can be predicted? Thus, considering the complexity of this system, it is highly unlikely that *in vitro* assays can replace *in vivo* screens for the foreseeable future. Still, *in vitro* assays may well be employed, with caution, to identify or eliminate specific mechanisms of action.

Considering the biology of thyroid hormone action in development, a number of conclusions can be made regarding our ability to develop a cogent battery of screens and tests that would effectively evaluate chemicals for the ability to interfere with thyroid hormone signaling. These conclusions are presented next, but the reader is strongly encouraged to refer to the background information presented in this document used in making these conclusions.

CONCLUSIONS

Several important conclusions can be derived from this detailed review article:

1. Research published in the past 5 years has clarified important issues germane to thyroid toxicology, and suggests endpoints and assays that should be considered for research and development and, if possible, current or future use in assay protocols (in addition to those initially recommended).
2. Many of the current *in vivo* screens and tests were originally designed to evaluate toxicant effects on reproduction and development. These protocols can be modified to test for thyroid toxicants by the addition of specific endpoints acquired at specific developmental time points. Although selected U.S. Environmental Protection Agency (EPA) and/or Organization for Economic Development (OECD) protocols are adequate in their dosing regimen and timing of treatment, they will require adaptation in the future for the timing of any newly developed thyroid endpoints designed to effectively evaluate toxicant effects on thyroid hormone action.
3. Thyroid hormones and thyroid histology are essential endpoints reflective of thyroid toxicity; in fact, all known thyroid toxicants have been identified by their ability to influence these endpoints. However, toxicants acting at different sites within the HPT axis appear to produce a different profile of hormone changes in relation to thyroid weight and histology. In addition, toxicant effects on the HPT axis may change over duration of treatment; thus, repeated sampling is important to capture dynamic events that may be informative.
4. Endpoints that measure the actions of thyroid hormones, both in development and in the adult, could greatly enhance the power of a data set generated on a specific thyroid toxicant to inform regulatory bodies. However, endpoints of thyroid hormone action are not well characterized within the context of toxicological research (i.e., dose sensitivity and specificity). Thus, these will require additional study before incorporated into existing screens and tests.
5. Thyroid endocrinology and biochemistry are remarkably conserved across vertebrate taxa (as discussed earlier).
6. A significant number of new reagents have become available, including identified genes and antisera, which will better support homologous assay development in nonmammalian vertebrates.

OVERALL STRATEGY FOR THYROID SCREENING AND TESTING

Thyroid assays using nonmammalian vertebrates can provide important information about potential thyroid toxicity in wildlife species. These assays may also have generalizable applicability to vertebrates considering the degree to which the thyroid system is conserved across taxa. Capturing endpoints of thyroid toxicity in preexisting *in vivo* rodent assays designed to evaluate reproductive and developmental toxicities provides the advantage of adding value to these assays without the use of additional animals. Moreover, careful design of the timing of toxicant exposure and thyroid endpoint acquisition in these assays can provide important information about the ability of specific toxicants to exert effects on development or on the adult by disrupting the thyroid system. This overall design feature has several implications as users of this document consider development of standardized approaches to evaluate thyroid toxicity and for the potential health consequences of these effects. If a tiered approach to thyroid toxicity testing is being considered, then the first line of screening should include measures of thyroid function, which represent the hallmark features of antithyroid actions of all known thyroid toxicants (Brucker-Davis, 1998). These measures include, in general, circulating levels of thyroid hormone and measures of thyroid histology. Thus, these endpoints should be incorporated into rodent assays designed to be part of an initial tier (e.g., acute studies). Chronic studies important to evaluate potential carcinogencity may have more apical endpoints (e.g., endpoints of thyroid hormone action) for added information. The second implication is that thyroid endpoints must be integrated into protocols in a manner that minimizes false-negatives. Thus, the following points should be considered when incorporating thyroid endpoints into existing experimental protocols.

A. Development of the HPT Axis

The HPT axis develops with a time course specific for the animal (across taxa). For example, metamorphosis in fish and amphibians represents a time when many changes are occurring, including changes in the sensitivity to thyroid hormone or in other hormones involved in regulation of the system. In rodents, the negative feedback action of thyroid hormone on the hypothalamus and pituitary does not fully develop until the first week

of life in the rat. Fukiishi and Hasegawa (1985) reported that rat fetal serum TSH concentration declined significantly between 20 and 21 days of gestation, reaching a low level at delivery, and remained low for several days after birth. T_3 suppressed serum TSH concentration further in a dose-responsive manner when given to fetuses on day 20 of gestation at 0.13 to 2.0 μg/100 g body weight of the estimated body weight. The responses of serum TSH levels and thyroid weights to PTU treatments differed with gestational age. Thus, they concluded that negative feedback control by T_3 of serum TSH concentration exists in rat fetuses as early as day 20 of gestation, but it differs from that found in adult rats. In addition, Taylor et al. (1990) found that thyroidectomy did not cause an increase in TRH mRNA levels of the hypophysiotropic PVN until PND 7, indicating that the hypothalamic limb of the negative feedback system developed later than that of the pituitary limb. Moreover, Nikrodhanond et al. (2006) have provided compelling evidence that the hypothalamus plays the dominant role (compared to negative feedback by TH) in regulating serum TH levels. Therefore, while thyroid endpoints of serum hormone levels and thyroid histology should be taken during the first postnatal week (e.g., PND 5 in OECD draft guideline 426), the interpretation of toxicant effects on these endpoints should take into consideration the development of the HPT axis.

B. Duration of Treatment

There are two competing issues when considering the duration of toxicant treatment and thyroid endpoints in vertebrates. The first is that because of the storage capacity of the thyroid gland, it may require several days before toxicant effects are observed on circulating levels of thyroid hormones and/or thyroid histology. In contrast, because of the potential compensatory mechanisms of the HPT axis and other tissue-level compensatory responses, thyroid toxicants may have a rapid effect on serum hormone levels that are "compensated" after some time. Moreover, it is clear that toxicants acting on the HPT axis through a different mechanism may elicit different compensatory responses and may require different durations. Considering this complexity of the HPT axis, thyroid endpoints should be captured at multiple time points. In adult rats, this may be represented by an early (48 hours) and late (14–28 days) time point. In development, this would be represented by two or three times during early postnatal development (e.g., P5, P15, and P21) as well as an adult time point.

C. Endpoints of Thyroid Function and Thyroid Hormone Action

Measures of thyroid function include serum hormone levels and thyroid histology. These endpoints represent the foundation of any assay for antithyroid activity. Changes in these measures of thyroid function associated with toxicant exposure represent the sole source of information by which thyroid toxicants have been identified, and hundreds of chemicals have been identified this way (see review by Bruker-Davis, 1998). There are a number of potential endpoints of thyroid hormone action, both in the adult and in the developing animal. These endpoints (Zoeller, 2006a, 2006b) could be utilized in experimental protocols at this time. However, more research and development would be needed before they are considered for validation in programs where needed.

ENDPOINTS OF THYROID HORMONE ACTION THAT REPRESENT POTENTIALLY USEFUL ENDPOINTS

The following overview includes endpoints and assays considered to be a priority for research and development as well as those available for validation, so that regulatory programs may further develop and/or incorporate those that will be most valuable for their particular purposes. For a specific list of existing or potential future assays, see Table 1. *In vitro* screens are described as potential ways to identify thyroid toxicants that act by very specific mechanisms (e.g., binding to TRs), that could be adapted to a high throughput platform. However, because of the complexity of regulation of the thyroid system, a very large and potentially unwieldy number of *in vitro* screens would have to be developed and employed to provide a comprehensive evaluation of all known mechanisms of thyroid toxicity. Moreover, because of the many points of regulation of thyroid endocrinology that can respond to fluctuations in the thyroid system, even these isolated *in vitro* screens would not be as comprehensive as an *in vivo* screen or test.

In Vitro Screening Assays

Research and Development

A number of *in vitro* screening assays are described in this article. Generally, these fall into two categories—*in vitro* systems that (1) specifically examine receptor binding and activation, and (2) allow observation of the consequences of disrupting specific modes of action. The following *in vitro* assays are in different states of research and development. None of them have been validated for use as screening assays, and all of them need various amounts of development before they could enter into validation.

In vitro thyroid hormone receptor (TR) binding and activation assays are equivalent to estrogen and androgen receptor binding and activation assays. They can be made to accommodate high throughput and can identify thyroid toxicants that interact directly with thyroid hormone receptors. All vertebrates have TRs; their comparative structure and the kinetics of T_3 binding to these TRs are quite similar. Therefore, it is theoretically possible that xenobiotics will bind to all vertebrate TRs with the same characteristics. This needs to be tested before being assumed.

In vitro assays that allow examination of thyroid hormone action may be useful, but certain disadvantages exist. For example, GH_3 cells may be used to detect generalized disruption of TR action in a manner analogous to the ESCREEN for estrogenic/antiestrogenic chemicals. Although this assay may be prone to false positives, it could be used as a tool to prioritize chemicals in conjunction with binding assays because these cells

TABLE 2
Points of disruption across taxa

Primary target	Mammals (Zoeller et al., 2006a)	Birds (McNabb, 2006)	Amphibians (Fort et al., 2006)	Fish (Blanton and Specker, 2006)
Thyroid				
Iodide uptake	Thyroid cells in all vertebrates, and cells of the endostyle in some invertebrates, concentrates iodide by the action of (at least) the sodium-iodide symporter (NIS). This protein is homologous in all vertebrates, but the comparative aspects of this protein in different vertebrates have not been well characterized. Therefore, while NIS inhibition is a potentially important point at which thyroid disruption could occur, research may show that specific chemicals (e.g., perchlorate) may be more potent in some vertebrates than in others. The protein Pendrin also may be an important target of toxicant actions. Pendrin in mammals is associated with iodine transport through the apical membrane into the region where peroxidase activity organifies the iodine. Likewise, Pendrin is expressed in all vertebrates and plays different roles in thyroid physiology.			
Iodine organification	The thyroperoxidase enzyme, similar to NIS, may be different enough among the taxa that it will respond differently to specific EDCs. Further research is required to clarify this issue. Moreover, TPO requires the activity of several enzymes to generate hydrogen peroxide in a location-specific manner. The DUOX proteins accomplish this in association with addition proteins that have not been well characterized. The comparative aspects of these proteins have not been well studied, and may be important in toxicological research.			
Thyroglobulin degradation	The initial solubilization of Tg occurs in the colloid by the combined actions of cathepsins B, L, and D. Further degradation occurs in secondary lysosomes as Tg is taken up by endocytosis and further degraded by cathepsins. Some exogenous factors are known to inhibit these enzymes—specifically lithium. However, the comparative aspects of these steps, which are present in all vertebrates, are poorly studied.			
Effects	The first effect of direct inhibition of thyroid function will be the reduction in thyroid hormone synthesis and secretion. There are a great many variables that differ among vertebrate taxa that will influence this effect. Specifically, differences among vertebrates in serum half-life for thyroid hormones, the storage capacity of the thyroid gland for thyroid hormone, and the relative sensitivity of thyroid hormone synthesis to EDCs acting on the NIS or TPO, will be important to consider when comparing the relative potency of EDCs among taxa.			
Hormone assays	In general, hormones are measured by radioimmunoassay (RIA). This is especially true for serum measurements and can also be true for tissue measurements, though additional physical measurements are also performed (e.g., HPLC, MS/GC). It is essential when considering the specific RIA, especially if this is a commercial kit, that the assay is validated. For thyroxine (T_4) and triiodothyronine (T_3), the chemistry of the hormone is identical among all vertebrates, but the specific matrix (e.g., serum) will be different. Therefore, it is possible—even common—that an RIA kit developed for humans will not be valid for use to measure the same hormone in the serum of other animals such as rodents, frogs, etc. Assay validation is accomplished by demonstrating that a dilution of matrix (e.g., serum) produces a linear function that is parallel to the standard curve. In addition, the addition of known quantities of hormone (e.g., T_4) to matrix (e.g., serum) should produce the expected results. Protein hormones such as TSH are most often not valid in heterologous assays in which the antibody is generated to a TSH from one species for use in another species. Thus, one should not predict that the rat TSH RIA will be valid for mouse. However, following the method just described for validation will demonstrate empirically whether the assay is valid or not. Finally, the standard curve should never be used between zero and the lowest standard (i.e., extrapolation). Rather, only interpolating between standards on a valid curve is appropriate. Thus, the specific RIA must be valid (i.e., parallel), but the standards must also be calibrated such that control animals are on the middle of the standard curve.			
Specific assays	RIA kits are commercially available for T_4, T_3, free T_4, free T_3, and TSH. The T_4 kit most commonly used (a human serum-based kit) is not well calibrated for T_4 in rats. Measurements of fT_4/fT_3 are vulnerable to changes in binding proteins and may be invalid. Volumes of serum required for the RIA can be large and therefore difficult to obtain in small animals (pups).	RIAs and ELISAs are in common use for thyroid hormones. Although T_4 and T_3 are chemically identical to thyroid hormones in all vertebrates, including humans, serum components may differ among taxa/species such that human kits are not valid. Validation procedures should be instituted. No immunoassay exists for avian TSH, but one could be developed. Serum volumes required for multiple assays often limiting.	RIAs and ELISAs are in common use for thyroid hormones. Although T_4 and T_3 are chemically identical to thyroid hormones in all vertebrates, including humans, serum components may differ among taxa/species such that human kits are not valid. Validation procedures should be instituted. No immunoassay exists for amphibian TSH, but one could be developed. Serum volumes required for multiple assays often limiting. Volumes available for analysis may be low and "whole-body" measures may be required.	RIAs and ELISAs are in common use for thyroid hormones. Although T_4 and T_3 are chemically identical to thyroid hormones in all vertebrates, including humans, serum components may differ among taxa/species such that human kits are not valid. Validation procedures should be instituted. No immunoassay exists for fish TSH, but this could be developed. Serum volumes required for multiple assays often limiting. Volumes available for analysis may be low and "whole-body" measures may be required.

Note: TSH is present as a protein dimmer in the pituitary of all vertebrate taxa. However, this large glycoprotein is different enough among taxa—and even between species within a class—that assays must be tailored for the specific TSH or a closely related one.

TABLE 2
Points of disruption across taxa *(Continued)*

Primary target	Mammals (Zoeller et al., 2006a)	Birds (McNabb, 2006)	Amphibians (Fort et al., 2006)	Fish (Blanton and Specker, 2006)
Thyroid measures	Thyroid gland weight and histopathology: May represent an integrated measure of thyroid function over time. Signs of hyperplasia may indicate susceptibility to cancer; however, this is controversial. Measure of stored T_4/T_3 not routinely performed but may be important.	Thyroid gland weight and histopathology: Both require training. Histopathology not validated for avian EDC research.	Thyroid structure differs from mammals and among amphibian species. Histopathology has not been validated for endocrine or EDC studies.	Thyroid structure differs from mammals and among fish species. Histopathology has not been validated for endocrine or EDC studies.
Adverse effects	Not routinely measured. Could include a variety of developmental and physiological endpoints. Developmental endpoints may be most sensitive. Potential assays are reviewed by Zoeller (2006a).	Not routinely measured. Could include a variety of developmental and physiological endpoints. Developmental endpoints may be most sensitive. Potential assays are reviewed in by McNabb (2006).	Amphibian metamorphosis being actively investigated as potential measure of EDC adverse effects on development. Many reagents/methods approaching validation.	Not routinely measured. Could include a variety of developmental and physiological endpoints. Flounder metamorphosis may be a simple and quantitative assay for EDC adverse effects through multiple modes of action.
Hormone metabolism				
Serum binding protein displacement	Yes	Yes	Yes	Yes
	Both TTR and thyroxine binding globulin (TBG) are present in mammals; TTR is present in all vertebrates. TTR does not appear to be developmentally regulated, or regulated by thyroid status, in mammals; however, TBG is very sensitive to thyroid status in adult rodents. The sensitivity of TBG expression in developing mammals is poorly understood, but may be important in toxicological studies. The role of T_4/T_3 displacement in the etiology of adverse effects induced by thyroid toxicants is suspected but not clear.			
Effects	A prevailing theory is that if T_4 (and/or T_3) is displaced from serum binding proteins, then the hormones will be more rapidly removed and adverse effects of thyroid hormone insufficiency will result. However, humans with defective or absent binding proteins have altered TH levels, but no symptoms of hypothyroidism. Moreover, TTR knockout mice have low serum hormone levels, but normal tissue levels (including brain). However, this mode of action may contribute to effects of EDCs on thyroid hormone levels. The three major thyroid hormone binding proteins—transthyretin, thyroxine-binding globulin, and albumin—are expressed in different ratios in different vertebrates and differ somewhat in their structure.			
Conjugation and glucuronidation	Glucuronidation and conjugation (sulfation and sulfonation) occur in all vertebrates and represent important pathways of T_4 degradation both in the liver and in target tissues. The enzymes required for accomplishing these steps have not been widely studied for their toxicological relevance. It is highly likely that induction of these enzymes will reduce serum thyroid hormones, but there is little evidence that xenobiotic-induced reduction of the expression or activity of these enzymes will have effects.			
UDPGT induction	Yes	Yes	Yes	Yes
Effects	Current theory is that induction of these enzymes by EDCs can increase their clearance (decreasing serum half-life) and causing adverse consequences mediated by thyroid hormone insufficiency. Evidence supports this concept, but there are UDPGTs selectively directed against T_4 or T_3 and EDCs may differ in their ability to induce one or both of these.			
Tissue uptake				
T_4 transporters	Yes	Yes	Yes	Yes
T_3 transporters	Yes	Yes	Yes	Yes
Effect	Several recent papers strongly suggest that T_3-transporters are expressed selectively on nerve cells within the central nervous system and that defects in this protein (MCT8) causes mental retardation and neurological deficits. Few endocrine or EDC studies have been performed, but these may be important.	Little information is available in birds for the existence of cellular transporters for T_3 and T_4. May be important site of EDC action.	There is some evidence that cells such as red blood cells have active TH transport in amphibians. Little work has been performed to identify these transporters and to characterize their importance in thyroid hormone signaling or as targets of EDC action.	More evidence exists for active transport mechanisms for cellular uptake in fish, but little evidence for the role of these proteins in physiology or effects of EDC on their function.

(Continued on next page)

TABLE 2
Points of disruption across taxa *(Continued)*

Primary target	Mammals (Zoeller et al., 2006a)	Birds (McNabb, 2006)	Amphibians (Fort et al., 2006)	Fish (Blanton and Specker, 2006)
TRs				
α/beta Isoforms	Yes	Yes	Yes	Yes
Effects	It is becoming clear that the different TR isoforms mediate different actions of thyroid hormone on development and physiologic of all vertebrates. There is more information available in mammals, but enough information exists in some representatives of other taxa to make this conclusion. This is important because there may be EDCs that selectively affect specific TR isoforms. Although this has not been identified for any EDC, it would complicate the identification of adverse effects because assays would have to be designed to identify TR isoform-specific endpoints. A second important issue is that while T_3 binds to all TRs, we do not know if individual EDCs bind to all TRs equally. This is not likely. Therefore, TR binding as an EDC screen may require TRs from different taxa to address this issue. Finally, the actions of TRs in different vertebrates are different. In addition, these actions differ at different life stages. Therefore, endpoints of EDC effects on TR actions must be strategically designed.			
Deiodinases—				
	There are two or three deiodinase enzymes in each taxon. These proteins share a great deal of similarity, but no studies have evaluated the effects of EDCs across the various deiodinases. However, considering that tissue expression of deiodinases controls sensitivity of the tissue to thyroid hormone, this may be an important point at which EDCs could disrupt thyroid hormone signaling .			
HPT axis	In all vertebrates, the dynamic relationships between the hypothalamus, pituitary and thyroid are functionally similar. Differences among vertebrates exist in some of the hypothalamic peptides controlling pituitary-thyroid function, but the HPT axis is functionally similar in all vertebrates.			

have both TRα and TRβ receptors and they respond to T_3 with proliferation.

Other *in vitro* assays allow the investigator to evaluate the effects of chemicals on specific modes of actions. Most of these assays use cell lines that can address specific modes of action of thyroid disruption. For example, FRTL-5 cells can be used for their ability to concentrate iodide. Purified thyroperoxidase or a crude extract can be used to test for the ability of chemicals to block this enzyme.

The *in vitro* assays are most useful in exploring specific modes of action, but it would be unrealistic to incorporate *in vitro* tests that cover all possible points of thyroid disruption across taxa into a screening and testing battery—a large battery of *in vitro* tests would have to be assembled to allow chemicals to be tested for all aspects of thyroid toxicity. Thus, it would appear to be most effective to focus on adapting existing *in vivo* assays for thyroid endpoints. As these would be added endpoints to existing assays, little or no increase in animal usage would be required.

Possible Inclusion in Validation at This Time

No *in vitro* assays are currently ready to validate in an existing screening battery. Several of the *in vitro* assays discussed in this document could be considered for validation after a limited amount of research and development.

In Vivo Screening Assays

Research and Development

It is important to recognize that all known thyroid toxicants (among hundreds) have been identified using endpoints of serum hormone levels and thyroid histology as endpoints of toxicity. Therefore, it is essential that these endpoints continue to be employed—and improved—to serve as bellwethers of thyroid toxicity. However, it is equally important to recognize that the pattern of changes in these endpoints of serum thyroid hormones and thyroid histology may not always be consistent with an idealized model of thyroid endocrinology. In these cases, and even perhaps in cases where the profile of endocrine changes are fully consistent with this idealized model, it may be of value to obtain measures of thyroid hormone action as further information on the adverse effects of the observed changes in circulating levels of thyroid hormone. Thus, the assays next are discussed as potential sites of thyroid hormone action that could be developed for such tier-2 studies.

In general, the *in vivo* screening assays are relatively short-term treatments of toxicants during peripubertal or adult life stages (e.g., OECD 407 and the male and female pubertal assays). In the future, simpler, less costly, and more informative endpoints may be developed to replace labor-intensive and expensive endpoints. For example, measuring thyroid gland T_4 content, as proposed by McNabb et al. (2004a, 2004b), may be a more sensitive indicator of TSH stimulation than current endpoints in the face of specific toxicants. Endpoints such as body weight or behavioral activities are affected by severe thyroid hormone insufficiency, but are not likely to be sensitive to small changes in circulating levels of thyroid hormones. There are few other *in vivo* endpoints of thyroid hormone action in adults that are well developed, and research in this area is needed.

Possible Inclusion in Validation at This Time

These assays can provide important information about thyroid toxicants if strategic endpoints are included as described in this document. As described earlier in this article, endpoints more relevant to thyroid hormone changes at different life stages, or to changes that occur following exposure to chemicals that

TABLE 3
Well-established endpoints of thyroid hormone action that may be recruited for toxicity studies

Specific endpoints	Mammals (Zoeller et al., 2006a)	Fish (Blanton and Specker, 2006)	Amphibians (Fort et al., 2006)	Birds (McNabb, 2006)
Brain			CNS restructuring: Restructuring of medulla and cerebellar neurons, genetically programmed regression/disappearance of giant neurons, Mauthner cells and Rohon–Beard neurons (Hughes, 1957; Moulton et al., 1968). Purkinje cells, lateral motor column neurons, and the dorsal root ganglia neurons further differentiate during metamorphosis (Hoskins, 1990)	
Genes	RC3/neurogranin	Few studies have focused on the effects of thyroid hormone on the fish brain. Thus, endpoints of TH action in fish brain are not available at this time.	Corticotropin-releasing factor (CRF): evidence supports a role for CRF in the regulation of TSH during metamorphosis (Denver et al., 2002; Okada et al., 2000).	Few studies have focused on the effects of thyroid hormone on the bird brain. Thus, endpoints of TH action in bird brain are not available at this time.
	Thyrotropin-releasing hormone		Thyroid-stimulating hormone (TSH): TSH genes have been cloned (cDNAs) in *X. laevis* (Buckbinder and Brown, 1993) encoding for both subunits and used as a diagnostic tool to measure the time course of expression through metamorphosis	
	Purkinje cell-specific protein-2		Thyroid hormone (TH) and thyroglobulin: biochemical measurements of glandular and plasma levels	
	Reelin		Other potential genes regulated by TH: Tail 1/3—zinc finger (BTEB), Xh20 (protein disulfide isomerase)	

(Continued on next page)

TABLE 3
Well-established endpoints of thyroid hormone action that may be recruited for toxicity studies *(Continued)*

Specific endpoints	Mammals (Zoeller et al., 2006a)	Fish (Blanton and Specker, 2006)	Amphibians (Fort et al., 2006)	Birds (McNabb, 2006)
	Hairless			
	Recent microarray studies reveal a large number of genes that are regulated by thyroid hormone, but many of these have not been pursued with focused hybridization studies.	It is important to recognize that there may be significant and important differences in the role of thyroid hormone in development and physiology among species within a single class of vertebrates. Therefore, as one considers developing endpoints of TH action in species that have not been used as model systems, it is possible that important differences among species will become apparent, rendering these efforts difficult to predict at the outset.		
		Flounder settling behavior (indicates effect on brain)		Type II deiodinase (not well established)
Developmental events	Cortical neuronal migration and establishment of cortical layers.	Flounder metamorphosis specific endpoints (e.g., eye migration) may be important.	Thyroid gland: development and histology during metamorphosis	Bone maturation
	Cerebellar development. Developmental timing of granule cell proliferation, migration across the mitral layer and survival/apoptosis in the internal granule layer.		Limbs: Hind limb differentiation and forelimb development and emergence	
	Cerebellar Purkinje cell arborization		Other metamorphic restructuring/resorption and biochemical changes: neurons, intestines, gills, lungs, tail. Biochemical changes also occur.	
	Myelination. Thyroid hormone plays a specific role in differentiation of oligodendrocytes and astrocytes from a common progenitor.		General rate of development: measured by development stage and hind limb length	

TABLE 3
Well-established endpoints of thyroid hormone action that may be recruited for toxicity studies *(Continued)*

Specific endpoints	Mammals (Zoeller et al., 2006a)	Fish (Blanton and Specker, 2006)	Amphibians (Fort et al., 2006)	Birds (McNabb, 2006)
	Cellular composition of bridging white matter (commissures, callosum).		Many TH upregulated genes directly linked with metamorphic events have been studied, including, but not limited to; stromelysin-3, TH/bZIP, and TRβ.	
Liver				
Genes	Malic enzyme		Potential genes found in late response to TH: carbamyl-phosphate synthetase I, arginosuccinate synthase and lyase, arginase, N-CAM, albumin	Malic enzyme gene expression/protein production in avian embryos (not well established)
	Alpha GPD		Type II and III deiodinase	
	Type I deiodinase		Metabolic changes: shift from ammonotelism to ureotelism	
	Thyroxin-binding Globulin (TBG)			
Heart				
Genes	SERCA-1		—	
	SERCA-2		—	
	MHC		MHC: In *X. laevis*, class I antigens are virtually absent from larval tissues until metamorphic climax (Rollins-Smith et al., 1997)	
Cardiovascular function	Heart rate		—	
	Blood pressure		—	
Tail				
Genes			Many TH up-regulated genes directly linked with metamorphic events have been studied	
Other endpoints				
		Flounder stomach formation (gastric glands)	CRF, which is the amphibian TRH	
			Genes in the metamorphosing tail (*Xenopus laevis*).	

alter thyroid hormone levels, could be added to existing *in vivo* assays with little alteration to the number of animals utilized.

In Vivo Tests

Research and Development

The *in vivo* tests include a number of developmental tests such as the OECD prenatal toxicity test or the one- or two-generation reproductive toxicity test. These tests can be modified to include measures of development that may be sensitive biomarkers of thyroid disruption. These future endpoints will likely be measures of histogenesis. There are a number of endpoints associated with neuronal differentiation and migration in the cerebellum and cerebral cortex (during cerebral cortical layering) in the developing brain. These endpoints may be highly sensitive to thyroid hormone insufficiency and would clearly reflect adverse effects. Endpoints for brain development are still progressing and are not yet ready for validation in any regulatory testing program.

Possible Inclusion in Validation at This Time

As described earlier (Zoeller et al., 2006b), additional time points for thyroid hormone measurement could accompany existing tests (such as the two-generation reproduction assay) so that developmental changes in thyroid hormone would be more accurately monitored.

Methods to Integrate Results from Multiple Species (Including Table 2, Showing Points of Disruption Across Taxa)

Interpreting results from several vertebrate taxa will provide useful information on cross-taxa similarities and differences. Two key considerations for interpretation of data are: (1) Different classes of vertebrates, and genera/species within those classes, likely have specific metabolic capacities or other physiological mechanisms that may render them particularly sensitive or insensitive to any one thyroid toxicant; and (2) it is likely that specific chemicals that interfere directly with thyroid hormone ssynthesis, transport, or signaling will exert these effects across vertebrate taxa; however, the specific effects of thyroid hormone (and disruption) in different taxa will vary considerably. We are just beginning to investigate these issues and we cannot expect to be able to derive broad inferences at this time.

IMPLICATIONS

The goal of this document is to provide a detailed review of the current literature of thyroid endocrinology and a basis for the strategic design of screens and tests to effectively identify environmental thyroid toxicants across taxa. The endocrine system is complex, and there are large gaps in our understanding of this system and the role it plays in development and physiology. Moreover, a reasonably comprehensive review of a variety of endpoints has been provided so that a broad perspective of available endpoints could be realized. The complexity of the endocrine system combined with large data gaps and endpoints uncharacterized in toxicological studies undoubtedly calls for ongoing research and development, as well as frequent re-evaluation and upgrading of the thyroid endpoints and assays used for regulatory purposes.

Table 1 shows existing or potential assays across all four taxa of interest, including a brief discussion of the strengths and weaknesses of each endpoint. Although reasonably comprehensive, the text provides a more complete discussion of the issues underlying these assays. Table 2 shows the primary targets of disruption across all four taxa with a brief discussion of their significance. Finally, Table 3 provides a listing of endpoints of thyroid hormone action that may be the most likely to be incorporated into various assays in the future based on current research.

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REFERENCES

Blanton, M.L., and Specker, J.L. (2006). The hypothalmic-pituitary-thyroid (HPT) axis in fish and its role in fish development and reproduction. *Crit. Rev. Toxicol.* **37**(1–2):97–115.

Brucker-Davis, F. (1998). Effects of environmental synthetic chemicals on thyroid function. *Thyroid* **8**:827–856.

Buckbinder, L., and Brown, D.D. (1993). Expression of the *Xenopus laevis* prolactin and thyrotropin genes during metamorphosis. *Proc. Natl. Acad. Sci. USA* **90**:3820–3824.

Denver, R.J., Glennemeier, K.A., and Boorse, G.C. (2002). Endocrinology of Complex Lifecycles: Amphibians. In: Hormones, Brain and Behavior, A.A. Pfaff, A. Etgen, S. Fahrbach, R. Moss, and R. Rubin, eds., pp. 469–513. Academic Press, San Diego.

DeVito, M., Biegel, L., Brouwer, A., Brown, S., Brucker-Davis, F., Cheek, A., Christensen, R., Colborn, T., Cooke, P., Crissman, J., Crofton, K., Doerge, D., Gray, E., Hauser, P., Hurley, P., Kohn, M., Lazar, J., McMaster, S., McClain, M., McConnell, E., Meier, C., Miller, R., Tietge, J., and Tyl, R. (1999). Screening methods for thyroid hormone disruptors. *Environ. Health Perspect.* **107**:407–415.

Fort, D. J., Dagitz, S., Tietge, J., Touart, L. W. (2006). The Hypothalmic-Pituitary-Thyroid (HPT) Axis in Frogs and Its Role in Frog Development and Reproduction. *Crit. Rev. Toxicol.* **37**(1–2):117–161.

Fukiishi, Y., and Hasegawa, Y. (1985). Ontogeny of thyrotrophin concentration in perinatal rats. *Acta. Endocrinol. (Copenh).* **110**:95–100.

Goldey, E.S., Kehn, L.S., Lau, C., Rehnberg, G.L., and Crofton, K.M. (1995). Developmental exposure to polychlorinated biphenyls (Aroclor 1254) reduces circulating thyroid hormone concentrations and causes hearing deficits in rats. *Toxicol. Appl. Pharmacol.* **135**: 77–88.

Hoskins, S.G. (1990). Metamorphosis of the amphibian eye. *J. Neurobiol.* **21**:970–989.

Hughes, A.F.W. (1957). The development of the primary sensory system in *Xenopus laevis* (Daudin). *J. Anat.* **91**:323–338.

McNabb, F.M.A. (2006). The hypothalmic-pituitary-thyroid (HPT) axis in birds and its role in bird development and reproduction. *Crit. Rev. Toxicol.* **37**(1-2):163–193.

McNabb, F.M.A., Larsen, C.T., and Pooler, P.S. (2004a). Ammonium perchlorate effects on thyroid function and growth in bobwhite quail chicks. *Environ. Toxicol. Chem.* **23**(4):997–1003.

McNabb, F.M.A., Jang, D.A., and Larsen, C.T. (2004b). Does thyroid function in developing birds adapt to sustained ammonium perchlorate exposure? *Toxicol. Sci.* **82**:106–113.

Moulton, J.M., Jurand, A., and Fox, H. (1968). A cytological study of Mauthner's cells in *Xenopus laevis* and *Rana temporaria* during metamorphosis. *J. Embryol. Exp. Morphol.* **19**:415–431.

Nikrodhanond, A.A., Ortiga-Carvalho, T.M., Shibusawa, N., Hashimoto, K., Liao, X.H., Refetoff, S., Yamada, M., Mori, M., and Wondisford, F.E. (2006). Dominant role of thyrotropin-releasing hormone in the hypothalamic-pituitary-thyroid axis. *J. Biol. Chem.* **281**(8):5000–5007.

OECD. (2006). Detailed Review Paper on Thyroid Hormone Disruption Assays. OECD Series on Testing and Assessment No. 57. ENV/JM/MONO(2006)24, Organisation for Economic Cooperation and Development (OECD) Environment Directorate, Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology, JT03212475, Available at http://www.oecd.org/dataoecd/49/35/37235405.pdf.

Okada, R., Iwata, T., Kato, T., Kikuchi, M, Yamamoto, K., and Kikuyama, S. (2000). Cloning of bullfrog thyroid-stimulating hormone (TSH) β subunit cDNA: Expression of TSHβ mRNA during metamorphosis. *Gen. Comp. Endocrinol.* **119**(2):224–231.

Rollins-Smith, L.A., Barker, K.S., and Davis, A.T. (1997). Involvement of glucocorticoids in the reorganization of the amphibian immune system at metamorphosis. *Dev. Immunol.* **5**:145–152.

Taylor, T., Gyves, P., and Burgunder, J.M. (1990). Thyroid hormone regulation of TRH mRNA levels in rat paraventricular nucleus of the hypothalamus changes during ontogeny. *Neuroendocrinology* **52**:262–267.

Zoeller, R.T., Dowling, A.L., and Vas, A.A. (2000). Developmental exposure to polychlorinated biphenyls exerts thyroid hormone-like effects on the expression of RC3/neurogranin and myelin basic protein messenger ribonucleic acids in the developing rat brain. *Endocrinology* **141**:181–189.

Zoeller, R.T., Tan, S.W., and Tyl, R.W. (2006a). General background on the hypothalamic-pituitary-thyroid (HPT) axis. *Crit. Rev. Toxicol.* **37**(1–2):11–53.

Zoeller, R.T., Tyl, R.W., Tan, S.W. (2006b). Current and potential rodent screens and tests for thyroid toxicants. *Crit. Rev. Toxicol.* **37**(1–2):55–95.